

The following Listing of the Claims will replace all prior versions and all prior listings of the claims in the present application:

Listing of The Claims:

1. (Currently amended) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme comprises a DNA 5'-3' polymerization activity of a DNA polymerase or reverse transcriptase, and said second enzyme is ~~a mutant~~ Pfu DNA polymerase consisting of comprising one or more mutations, wherein said mutation(s) are an amino acid substitution at an amino acid position selected from amino acid positions selected from the group consisting of: Y410, T542, D543, K593, Y595, Y385, G387, and G388, and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity.
2. (Original) The enzyme mixture of claim 1, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.
3. (Currently amended) The enzyme mixture of claim 2, wherein said first enzyme ~~DNA polymerase~~ is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UITma DNA polymerase, Tli DNA polymerase (Vent DNA polymerase), Pwo DNA polymerase, Tgo DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase having the sequence shown in SEQ ID NO. 10, PGB-D DNA polymerase (Deep Vent DNA polymerase) and DP1/DP2 DNA polymerase.
- 4-9. (Cancelled)
10. (Currently amended) The enzyme mixture of claim 1, wherein said ~~mutant~~ Pfu DNA polymerase consists of comprises one or more amino acid substitutions mutations selected from the group consisting of: Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.
11. (Previously presented) The enzyme mixture of claim 1, further comprising a PCR enhancing factor and/or an additive.

12. (Currently amended) A kit comprising a first enzyme, a second enzyme, and packaging material therefor, wherein said first enzyme comprises 5'-3' a DNA-polymerization activity of a DNA polymerase or reverse transcriptase, said second enzyme is ~~a mutant~~-Pfu DNA polymerase consisting of comprising one or more mutations, wherein said mutation(s) are an amino acid substitution at an amino acid position selected from amino acid positions selected from the group consisting of: Y410, T542, D543, K593, Y595, Y385, G387, and G388, and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity.

13. (Original) The kit of claim 12, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

14. (Currently amended) The kit of claim 13, wherein said first enzyme ~~DNA polymerase~~ is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UITma DNA polymerase, Tli DNA polymerase (Vent DNA polymerase), Pwo DNA polymerase, Tgo DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase having the sequence shown in SEQ ID NO. 10, PGB-D DNA polymerase(Deep Vent DNA polymerase) and DP1/DP2 DNA polymerase.

15-19. (Cancelled)

20. (Previously presented) The kit of claim 12, further comprising one or more components selected from the group consisting of: a deoxynucleotide, a reaction buffer, a PCR enhancing factor and/or an additive, a control DNA template and a control primer.

21. (Cancelled)

22. (Currently amended) The kit of claim 12, wherein said ~~mutant~~-Pfu DNA polymerase consists of comprises one or more amino acid substitutions ~~mutations~~ selected from the group consisting of: Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

23. (Withdrawn) A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a first enzyme comprising a DNA polymerization activity, and a second enzyme which is a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388; and

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

24. (Withdrawn) The method of claim 23, wherein said nucleic acid template is a DNA molecule.

25. (Withdrawn) The method of claim 24, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

26. (Withdrawn) The method of claim 25, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UITma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

27-29. (Cancelled)

30. (Withdrawn) A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a wild type Pfu DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity; and

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

31. (Withdrawn) A method for TA cloning of DNA synthesis product comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a Taq DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity;

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis to generate a synthesized DNA product; and

(c) inserting said synthesized DNA product into a TA cloning vector.

32. (Withdrawn) The method of claim [28, ]30, or 31, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

33. (Withdrawn) The method of claim 23, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

34. (Withdrawn) The method of claim 23, 30 or 31, wherein said reaction mixture further comprises a PCR enhancing factor and/or an additive.

35. (Withdrawn) The method of claim 32, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

36. (Currently amended) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is Taq DNA polymerase, and said second enzyme is ~~a mutant~~ Pfu DNA polymerase consisting of comprising an amino acid substitution at an amino acid position ~~one or more mutations, wherein said mutation(s) are~~ selected from amino acid positions selected from the group consisting of: Y410, T542, D543, K593, Y595, Y385, G387, and G388, and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity.

37. (Currently amended) The enzyme mixture of claim 36, wherein said ~~mutant~~ Pfu DNA polymerase consisting of comprises one or more amino acid substitutions ~~mutations~~ selected

from the group consisting of: Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

38. (Currently amended) The enzyme mixture of claim 36, wherein said ~~mutant~~ Pfu DNA polymerase consists of ~~comprises~~ an amino acid substitution ~~mutation~~ at amino acid position G387.

39. (Currently amended) The enzyme mixture of claim 36, wherein said ~~mutant~~ Pfu DNA polymerase consists of ~~comprises~~ the amino acid substitution ~~a mutation~~ of G387P.

40. (Currently amended) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is KOD DNA polymerase, and said second enzyme is a ~~mutant~~ Pfu DNA polymerase consisting of ~~comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions~~ an amino acid substitution at an amino acid position selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388, and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity.

41. (Currently amended) The enzyme mixture of claim 40, wherein said ~~mutant~~ Pfu-DNA polymerase comprises one or more amino acid substitutions ~~mutations~~ selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

42. (Currently amended) The enzyme mixture of claim 40, wherein said ~~mutant~~ Pfu DNA polymerase comprises an amino acid substitution ~~mutation~~ at amino acid position G387.

43. (Currently amended) The enzyme mixture of claim 40, wherein said ~~mutant~~ Pfu DNA polymerase comprises the amino acid substitution ~~mutation~~ of G387P.

44. (Currently amended) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a JDF-3 DNA polymerase having the sequence shown in SEQ ID NO. 10, and said second enzyme is a ~~mutant~~ Pfu DNA polymerase consisting of ~~comprising one or more mutations, wherein said mutation(s) are~~ an amino acid substitution at an

amino acid position selected from ~~amino acid positions selected from~~ the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388, and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity.

45. (Currently amended) The enzyme mixture of claim 44, wherein said ~~mutant~~ Pfu-DNA polymerase consists of ~~comprises~~ one or more amino acid substitutions ~~mutations~~ selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

46. (Currently amended) The enzyme mixture of claim 44, wherein said ~~mutant~~ Pfu-DNA polymerase consists of ~~comprises~~ an amino acid substitution ~~mutation~~ at amino acid position G387.

47. (Currently amended) The enzyme mixture of claim 44, wherein said ~~mutant~~ Pfu DNA polymerase consists of ~~comprises~~ a the amino acid substitution ~~mutation~~ of G387P.

48. (Currently amended) A kit comprising a first enzyme and a second enzyme, wherein said first enzyme is a Taq DNA polymerase, and said second enzyme is ~~a mutant~~ Pfu DNA polymerase consisting of ~~comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions~~ an amino acid substitution at an amino acid position selected from the group consisting of: Y410, T542, D543, K593, Y595, Y385, G387, and G388, and packaging material therefor, and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity.

49. (Currently amended) A kit comprising a first enzyme and a second enzyme, wherein said first enzyme is a KOD DNA polymerase, and said second enzyme is ~~a mutant~~ Pfu DNA polymerase consisting of ~~comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions~~ an amino acid substitution at an amino acid position selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388, and packaging material therefor, and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity.

50. (Currently amended) A kit comprising a first enzyme and a second enzyme, wherein said first enzyme is a JDF-3 DNA polymerase having the sequence shown in SEQ ID NO. 10, and said second enzyme is a ~~mutant~~ Pfu DNA polymerase consisting of comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions an amino acid substitution at an amino acid position selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388, and packaging material therefor, and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity.

51. (Previously Presented) The kit of claim 48, 49, or 50, wherein said kit further comprises a reagent selected from the group consisting of: dNTPs, reaction buffer, primer, and DNA enhancing factor.